

What is claimed is:

1. A method for monitoring nucleic acid amplification comprising:
performing nucleic acid amplification on a target polynucleotide wherein the
amplification is carried out using any method using a first oligonucleotide probe and a
second shorter oligonucleotide probe varying in length by at least about 2 base pairs;
the first probe having a fluorophore;
the second being complementary with the first probe and having a quencher
molecule capable of quenching the fluorescence of said fluorophore, the fluorophore
and quencher being attached on their respective probes at positions which results in the
quencher molecule quenching the fluorescence of the fluorophore when the probes are
hybridized,
wherein the longer probe binds preferentially to the target polynucleotide and
when preferentially bound to the target polynucleotide the fluorescence intensity of the
fluorophore is greater than the fluorescence intensity of the fluorophore when
hybridized to the second probe, and
monitoring the fluorescence of the fluorophore, the generation of fluorescence
corresponding to the occurrence of nucleic acid amplification.
2. The method of claim 1 wherein the nucleic acid polymerase is a
thermostable nucleic acid polymerase.
3. The method of claim 1 wherein the fluorophore on the first probe and
the quencher molecule on the second probe are on the same hybridized base pair.
4. The method of claim 1 wherein the fluorophore and quencher molecules
are within about 1 to 3 hybridized base pairs of each other.
5. The method of claim 1 wherein the fluorophore and quencher molecules
are within 3 or more hybridized base pairs of each other.
6. The method of claim 1 wherein the fluorophore is on the 5' terminal
nucleotide of the first probe and the quencher is on the 3' terminal nucleotide of the
second probe.
7. The method of claim 1 wherein the fluorophore is on the 3' terminal
nucleotide of the first probe and the quencher is on the 5' terminal nucleotide of the
second probe.
8. The method of claim 1 wherein the second probe is shorter than the first
probe by deletion of 3 or 3' terminal nucleotides from the nucleotide sequence of the
first probe.
9. The method of claim 1 wherein the second probe is shorter than the first
probe by deletion of 3 or more 3' terminal nucleotides from the nucleotide sequence of
the first probe.

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11. The method of claim 1 wherein the second probe is shorter than the first probe by deletion of 3 or more 5' terminal nucleotides, and deletion of 1 or more 3' terminal nucleotides of the first probe.
12. The method of claim 1 wherein the first and second probes have a
5 disassociation temperature difference of 2 degrees or more.
13. A method for detecting the presence of specific nucleic acid sequences in a prepared nucleic acid sample comprising:
placing a sample of nucleic acids in a suitable solution and incubating with a first oligonucleotide probe and a second shorter oligonucleotide probe varying in length
10 by about at least 2 base pairs;
the first probe having a fluorophore;
the second being complementary with the first probe and having a quencher molecule capable of quenching the fluorescence of said fluorophore, the fluorophore and quencher being attached on their respective probes at positions which results in the
15 quencher molecule quenching the fluorescence of the fluorophore when the probes are hybridized,
wherein the longer probe binds preferentially to the target polynucleotide and when preferentially bound to the target polynucleotide the fluorescence intensity of the fluorophore is greater than the fluorescence intensity of the fluorophore when
20 hybridized to the second probe, and
monitoring the fluorescence of the fluorophore, the generation of fluorescence corresponding to the presence of specific nucleic acid sequences.

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Table I

CV 03 SEQ

| | | | | | | | | | |
|------|--------------|--------------|------------|------------|-------------|-------------|------------|------------|------------|
| N | 61 | 71 | 81 | 91 | 1 | 11 | 21 | 31 | |
| 3151 | SGGDIYHSVS | HARPRWFEC | LLLLAAGVGI | YLLPNRBASE | CNTACGTIRIG | INGCCAGCCC | CCTGATGGGG | GCGACACTCC | ACCATGAATC |
| N | 51 | 61 | 71 | 81 | 91 | 1 | 11 | 21 | |
| 3241 | ACTCCCCTGT | GAGGAACACTAC | TGTCTTCACG | CAGAAAGCGT | CTAGCCATGG | CGTTAGTATG | AGTGTCTGTC | AGCCTCCAGG | ACCCCCCTC |
| | | | | | | | | | |
| | | | | | | | | | |
| N | 41 | 51 | 61 | 71 | 81 | 91 | 1 | 11 | |
| 3331 | F CCGGGAGAGC | CATAGTGGTC | TGCGGAACCG | GTGAGTACAC | CGGAATTGCC | AGGACGACCG | GGTCCTTTCT | TGGATAAAC | CGCTCAATGC |
| | | | | | | | | | |
| | | | | | | | | | |
| N | 31 | 41 | 51 | 61 | 71 | 81 | 91 | 1 | |
| 3421 | CTGGAGATTT | GGGCGTGCCC | CCGCAAGACT | GCTAGCCGAG | TAGTGTGGG | TCGCGAAAAGG | CCTTGTGGTA | CTGCCTGATA | GGGTGCTTGC |
| | | | | | | | | | |
| N | 21 | 31 | 41 | 51 | 61 | 71 | 81 | 91 | |
| 3511 | GAGTGCCCCG | GGAGGTCTCG | TAGACCGTGC | ACCATGAGCA | CGAATCCTAA | ACCTCAAGA | AAACCAAAC | GTAACACCAA | CCGTGCCCCA |

Probes (C1, C2)

Primer F

Primer R

Probes (C1, C2)

← T
 CATAGTGGTC
 TGCCTGCTCTC

Primer R

Primer F